LuxScan™ 10K

Microarray Scanner

User Manual

Version 3.0

CapitalBio Corporation

End User License Agreement

IMPORTANT-PLEASE READ CAREFULLY:

This End User License Agreement (hereinafter referred to as “Agreement”) is a valid and binding agreement between CapitalBio Corporation (hereinafter referred to as “CapitalBio”) and you (either an individual or a single entity) for the use of LuxScan™ 10K Microarray Scanner Application Software System (hereinafter referred to as “System”).

This Agreement is an indispensable part of the documents of this System. You have the right to require the whole documents from the seller of this System for protecting your rights and interests.

This System consists of computer software, including relevant media, printed materials and electronic documents. This System may also include any updated or upgraded software and supplemental materials, which are provided by CapitalBio for the use of the original System. This System and relevant documents shall be granted to you in accordance with this Agreement. By installing, copying, downloading, or using this System in any way, you agree to be bound by the terms of this Agreement. If you do not agree to be bound by the terms of this Agreement, please do not install or use this System, but you may return it to where you bought it and refund your payment.

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3. You can only use this System in a multi-user environment or a network system under one of the following conditions:
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   you have purchased the use license for every related computer.

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Post Sales Warranty:

CapitalBio warrants that within ninety (90) days after the selling of this System, this System shall not have any material

defects or technical defects, subject to the condition that this System is used in its regular way.
In the event that the defect is confirmed by CapitalBio, CapitalBio shall only be responsible for replacing the defected software, and this is the only remedy for you.

If the defect is caused by or arising from any accident, abuse, or mistake, the above mentioned post sales warranty shall be invalid.

Exemption from Liability:

Except for the contents specified in the above paragraphs, you shall not have any other post sales warranties of this System.

Limitation of Liability:

Subject to the scope of applicable laws specified in this Agreement, in no event shall CapitalBio and its agents and sales persons be liable for any special, incidental, indirect, or consequential damages and/or losses (including but not limited to profit loss, operation interruption, business interruption, business information loss and any other pecuniary loss) arising from the use of this System or inability to use this System.

CapitalBio does not guarantee the stability of this System, if you install other software in the process of using this System on the same computer.

This system may only work and function regularly under correct usage.

CapitalBio's total liability to you for any damages and/or losses for any causes whatsoever under the terms of this Agreement will be limited to the actual amount paid by you for this System.

Termination of License:

If you breach any term or condition of this Agreement, CapitalBio shall have the right to terminate this license at any time. If CapitalBio decides to terminate this license, you must immediately destroy (no more than three (3) days after the termination) this System and any copies of any documents of this System or return them to CapitalBio.

Applicable Laws:

This System shall be protected by Copyright Law of the People's Republic of China and relevant international intellectual property protection treaties, which have been acceded to and enforced in the People's Republic of China.

This Agreement shall be governed by the law of the People's Republic of China.

If you have any question with respect to this Agreement, please contact CapitalBio: bd@capitalbio.com.

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Preface

Welcome to the LuxScan™ 10K Microarray Scanner 3.0 User Manual. Users should read this manual carefully, follow the instructions and procedures, and be aware of all the safety information. Make sure the system that you are using is the CapitalBio Corporation LuxScan™ 10K Microarray Scanner 3.0.

Contents of This Manual

Chapter	Description
Important Safety Information	Provides crucial information to use the system safely. All LuxScan™ 10K users should read this chapter before installing and using the system.
1. Hardware Installation	Provides LuxScan™ 10K Microarray Scanner hardware specifications and the step-by-step instructions for installation of the system.
2. Software Installation	Provides instructions on how to install the LuxScan™ 10K application software and the device driver.
3. Tutorial	Provides instructions on how to scan a microarray chip and analyze the image.
4. Additional Software Features	Provides description of additional software features.
5. System Maintenance	Provides information on routine maintenance for quality service.
6. Troubleshooting	Provides answers to frequently asked questions and solutions to frequently encountered problems.

Note:

There are two types of LuxScan™ 10K Microarray Scanner product: LuxScan™ 10K-A (Catalog Number: 100010) dual laser scanner and LuxScan™ 10K-B (Catalog Number: 100020, 100030) single laser scanner. The LuxScan™ 10K-A Microarray Scanner can scan dual channel images of a microarray slide and the LuxScan™ 10K-B Microarray Scanner can only scan one channel.

‚All features in this manual involving dual laser operation are only available on the LuxScan™ 10K-A scanner.

Conventions

In this manual, the names of keys, buttons and menu items are **bold**, and the names of windows, dialog boxes and controls are in *italics*. For example:

- Click the **Align All** button on the *Tool Bar* or press **F6** key to align all *blocks* on the image.

All user defined file names must be Microsoft Windows® operating system compatible (i.e. they cannot contain a slash, a backslash, a dot or any other operating system reserved characters).

We recommend someone familiar with the Microsoft Windows® 2000/XP administration to install the software onto the computer workstation.

Where to Get Help

Our staff at CapitalBio Corporation wants to help with any questions or comments you might have regarding the system, its software and other components. Feel free to contact CapitalBio Corporation in the following ways:

- Address: 18 Life Science Parkway Changping District, Beijing 102206, China
- Web Site: www.capitalbio.com
- Main: 86-10-80726868
- Technical Support: 86-10-80725793
- Fax: 86-10-80726782
- Email (General Info): bd@capitalbio.com
- Email (Technical Support): techsupport@capitalbio.com

Please prepare the following information for optimum technical support:

- serial number of your LuxScan™ 10K Microarray Scanner (at the back of the scanner)
- version number of the application software
- nature of the problem
- steps that you have taken to correct the problem
- your name, your phone number, fax number, e-mail address, and street address if necessary
- your normal form of address if contacting us by fax or email

In addition, convenient access to your LuxScan™ 10K Microarray Scanner during the duration of a technical support call would facilitate the process.

Additional information can be found in the following:

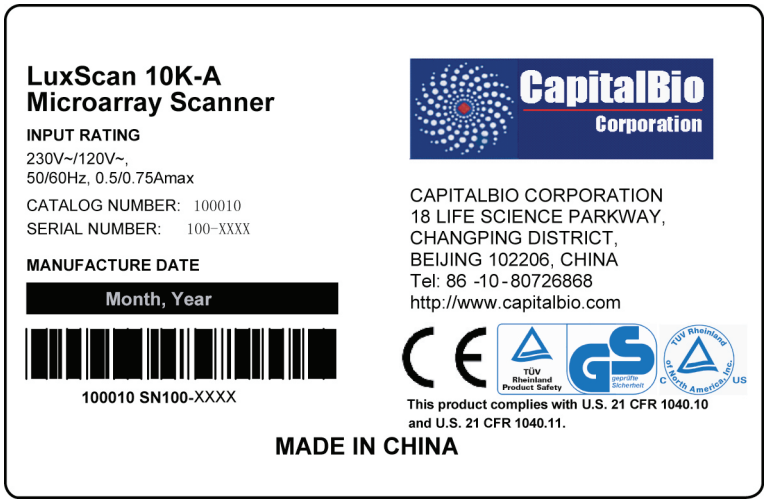
- Documentation provided with the computer workstation and its software

Important Safety Information

Warning:

Use of controls or adjustments or performance of procedures other than those specified in the user manual may result in damage to the instrument, loss of data, invalid data, hazardous radiation exposure, expose laser light, hazardous voltage, or moving parts, or even cause fire. Exposure to the hazards may cause severe or fatal injury.

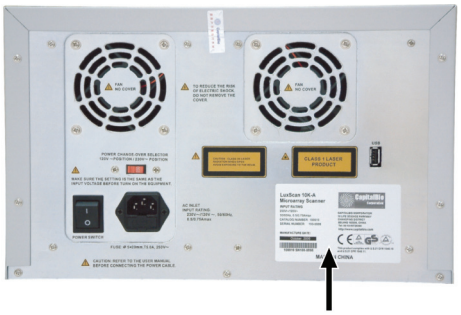
1. Certification, Identification and Power Supply Label



Certification, Identification and Power Supply Label

The SERIAL NUMBER 100-XXXX and SN100-XXXX are only examples to describe the label, and XXXX represents four digitals such as 0123, and would be different from the numerals on the label of your LuxScan™ 10K Microarray Scanner.

Rear panel of the LuxScan™ 10K Microarray Scanner:



Label for Certification, Identification and Power Supply

The LuxScan™ 10K Microarray Scanner is designed to comply with the following EC Council Directives and product safety standards:

Application of Council Directives:

- EC Council Directive 89/326/EEC as last amended by EC Directive 93/68/EEC ELECTROMAGNETIC COMPATIBILITY
- Low Voltage Directive 73/23/EEC as last amended by EEC Directive 93/68/EEC

Application of Harmonized Standards:

- EN 61326:1997+A1+A2
- EN 61000-3-2:2000
- EN 61000-3-3:1995+A1
- EN 61010-1:2001
- EN 61010-2-081:2002
- IEC 60825-1:1993+A1:1997+A2:2001/EN60825-1:1994+A1:2002+A2:2001
- UL 61010-1:2004
- CAN/CSA-C22.2 61010-1:2004
- CAN/CSA-C22.2 61010-2-081:2004

Application of U.S. PERFORMANCE STANDARDS FOR LIGHT-EMITTING PRODUCTS:

- US 21 CFR 1040.10
- US 21 CFR 1040.11

2. Classification of the Scanner

The LuxScan™ 10K Microarray Scanner complies with safety standard IEC 60825-1:1993 +A1:1997 +A2:2001/EN60825-1:1994 +A1:2002 +A2:2001 for a Class 1 laser product. It also complies with U.S. 21 CFR 1040 Class as applicable to a Class I laser product.

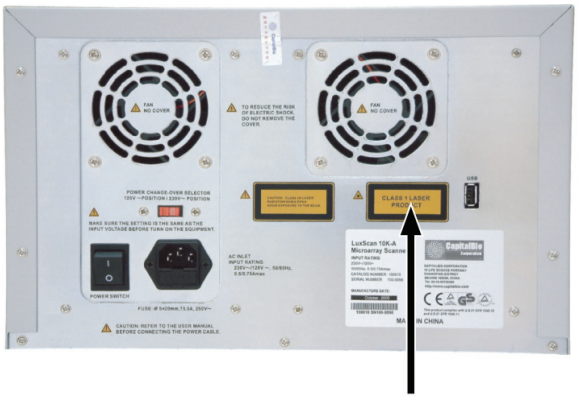
Classification of the Scanner

(Complies with U.S. Market Requirements)

Classification of the Scanner

(Complies with EUROPEAN UNION Market Requirements)

Rear panel of the LuxScan™ 10K Microarray Scanner rear panel:



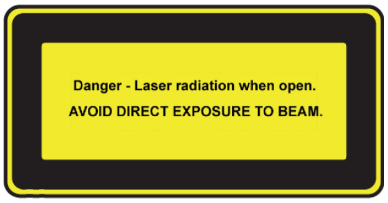
Label for Classification of the LuxScan 10K Microarray Scanner

3. The protective housing

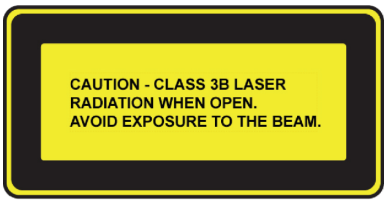
The protective housing is designed to protect users from exposure to the laser radiation, laser light, hazardous voltage, or moving parts. Users are not allowed to access the interior of the LuxScan™ 10K Microarray Scanner. There is no component in the interior of the LuxScan™ 10K Microarray Scanner that requires maintenance work to be performed by the user. Users are not allowed to remove the cover of the LuxScan™ 10K Microarray Scanner, otherwise, warranty on the product will be voided with immediate effect.

	Warning: Users are not allowed to gain access to the interior of the LuxScan™ 10K Microarray Scanner through any other openings except that is needed to perform the function of the LuxScan™ 10K Microarray Scanner. Removing the housing may damage the LuxScan™ 10K Microarray Scanner components and result in hazardous exposure to laser radiation, laser light, hazardous voltage, or moving parts. If the protective housing is damaged, users are instructed to halt all use until a certified technician has inspected the instrument.

The protective housing of LuxScan™ 10K Microarray Scanner is not interlocked and is fastened on the rear panel with screws. Do not remove any screws on the rear panel.

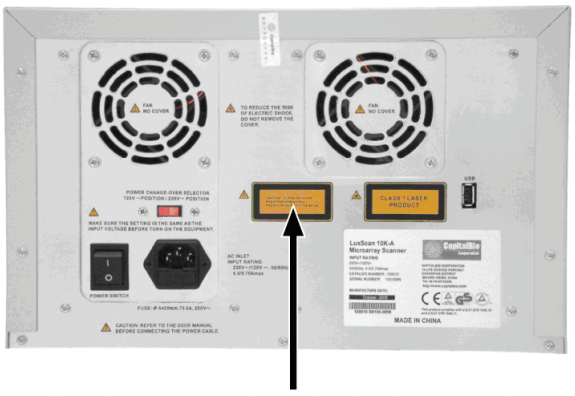


(Complies with U.S. Market Requirements)



(Complies with EUROPEAN UNION Market Requirements)

Location on the LuxScan™ 10K Microarray Scanner:



Label Indicating a Non-interlocked Protective Housing

4. Lasers

There are two Class IIIb lasers embedded inside the LuxScan™ 10K Microarray Scanner, which are inaccessible to the user. Slides may be put safely into the scanner by inserting into the chip entrance located on the front panel. The protective housing can protect users from exposure to laser radiation.

Laser specifications:

Wavelength (nm)	Power (mW)	Beam Divergence (mrad)	Beam Diameter at Laser Aperture (mm)	Operation Mode	Class
635	25	1	1	Continuous	IIIb
532	20	1.1	1.1	Continuous	IIIb

5. Power Change-over Selector

The LuxScan™ 10K Microarray Scanner can be used with power sources at either 120V alternating current at a frequency of 50/60Hz, or 230V alternating current at a frequency of 50/60Hz. **Set the Power Change-over Selector to the correct position for the input voltage in your area**. Users must ensure that the power setting of the LuxScan™ 10K Microarray Scanner is in the correct position. Operation in the incorrect setting may result in electric shock, or cause fire and damage the scanner.

Warning:

Make sure the power setting is the same as the rated input voltage.

6. Power Cable (Cord)

The manufacturer of the LuxScan™ 10K Microarray Scanner provides the user with the power cable (cord). The cord is PVC insulated (area of 0.75 mm^2), and is usable for voltages up to and including 450V/750V. Users must not to replace the power cable with one of a different rating, or it may result in electric shock, or cause fire and damage the scanner.

Warning:

We suggest that users do not replace the power cable (cord) provided by the manufacturer with another type. If a replacement cable (cord) is needed, contact the technical support team for the LuxScan™ 10K Microarray Scanner. Replacing the power cable with another cable of a different rating may result in electric shock or cause fire and damage the scanner.

7. Fuses

The LuxScan™ 10K Microarray Scanner contains two fuses, the rating of which is 5mm(diameter)×20mm(height), T5.0A, 250V~. Spent fuses can be replaced by users. Switch off the electricity supply at source, or remove the power connection from the socket before removing or replacing fuses. There is a marking beside the fuse-holder which is in the appliance inlet (AC INLET). Users must replace the fuses with the same type and rating. If instructions are not followed, it may result in electrical hazard or cause a potential fire.

	Warning: Replace only with same type and rating fuses, otherwise it may result in an electrical hazard or even dangerous fire. Switch off the electricity supply at source or remove power connection from socket before removing or replacing fuses.

8. Fan

Two fans exist in the LuxScan™ 10K Microarray Scanner for the purpose of ventilation. Users are not allowed to block or cover the vent of the fans. If vents are blocked the temperature will rise and result in damage to the scanner, and possibly cause electric shock or fire.

	Warning: Users are not allowed to cover the vent of the fans in the rear panel of the LuxScan™ 10K Microarray Scanner. Otherwise the temperature will rise and result in damage to the scanner; possibly causing electric shock and fire.

9. Moving Parts

The LuxScan™ 10K Microarray Scanner contains moving parts which may lead to a mechanical hazard if users do not follow the instructions manufacturer provided, or moving parts may cause injury to users. The protective housing is designed to protect users from accessing the moving parts during operation.

	Warning: Users are not allowed to gain access to the interior of the scanner during operation. The protective housing of the scanner is designed to protect users from the hazardous moving parts under operation.

10. Service

Service is conducted by the technical support team of CapitalBio Corporation or authorized agencies only. Consult CapitalBio Corporation or authorized agencies for repair instructions. See “Where to Get Help” in the Preface for more information.

Chapter 1 Hardware Installation

1.1 Overview

The LuxScan™ 10K Microarray Scanner is designed to be installed by the user. Before starting hardware installation, the user should read this chapter carefully, and understand all the requirements and steps. The user should always follow the instructions during installation, in order to avoid causing any damage to the LuxScan™ 10K scanner.

1.2 Requirements

1.2.1 Environment

The LuxScan™ 10K Microarray Scanner should be installed in a facility that meets the following environmental conditions:

Site Requirement	Indoor Use
Temperature Range	5 – 40 °C (41 – 104 ^{o}F)
Relative Humidity Range	Up to 80 %
Altitude	Up to 2000 m

Because the scanner is a precision optical instrument, please keep the environment as clean as possible. Install the scanner away from windows, ventilation ducts and any other devices that may cause significant change in temperature and humidity.

1.2.2 Space

The dimensions of the LuxScan™ 10K Microarray Scanner are 540mm×360mm×220mm. The scanner should be placed on a bench, which can support at least 50kg (110lb) of weight. The control computer should be placed near the scanner within the length of the provided USB cable, but should not be in immediate contact with the scanner.

1.2.3 Power

The total power consumption of the system is less than 75W and the maximum input current is 0.75A for 120V~ 50/60Hz power supply and 0.5A for 230V~ 50/60Hz power supply.

1.2.4 Electromagnetic Compatibility (EMC)

An appropriate EMC environment is critical to the LuxScan™ 10K scanner operation, since large noise fluctuations may lead to unpredictable results. The following precautions should be enacted:

- Keep the scanner away from high electrical discharge equipment, such as pacemakers, electric welding equipment, etc.
- Keep the scanner away from frequently starting-up, high power-consumption equipment, such as refrigerators, centrifuges, etc.
- Keep the scanner away from any strong magnetic field.
- Do not connect too many power cables to the same junction box to which the scanner is connected.
- Do not plug-in or unplug any other electrical equipment to the same junction box, while the scanner is running.

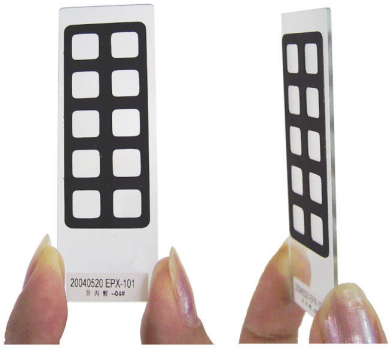
1.2.5 Computer Workstation

To install the LuxScan™ 10K application software and run common tasks smoothly, the computer should meet the following minimum requirements:

CPU	P4 2.0G or higher
RAM	512M or more
Virtual Memory	512M or more
Disk Space	40G or more
CD-ROM Drive	40X or faster
USB Controller	USB 2.0
Display System	17’ color display 1024×768 pixels
Operating System	Microsoft Windows® 2000 (with Service Pack 4), or Microsoft Windows® XP (with Service Pack 1 or higher)

1.2.6 Chip Dimensions

The dimensions of a chip to be scanned by the LuxScan™ 10K scanner should meet the following requirements:



Length	76.20±0.50 mm
Width	25.40±0.25 mm
Thickness	1.00±0.10 mm
Flatness	±20 μm
Cover Width	< 23 mm
Cover Thickness	< 0.8 mm
Barcode Width	< 23 mm

1.3 Receiving Inspection

1.3.1 Initial Inspection

If any external damage is noted, please indicate the damage on the shipping receipt when you sign for the system. If any internal or concealed damaged is noted, after the carton is opened, please contact both the shipping agent and CapitalBio Corporation immediately.

Note:

If there is any apparent damage to the system, please do not plug it into the power source.

1.3.2 Unpacking

Note:

Care should be taken when unpacking the system or placing it on a laboratory bench. Shocks may damage or misalign optical components of the scanner. It is recommended to have two persons to lift the scanner out of the shipping carton.

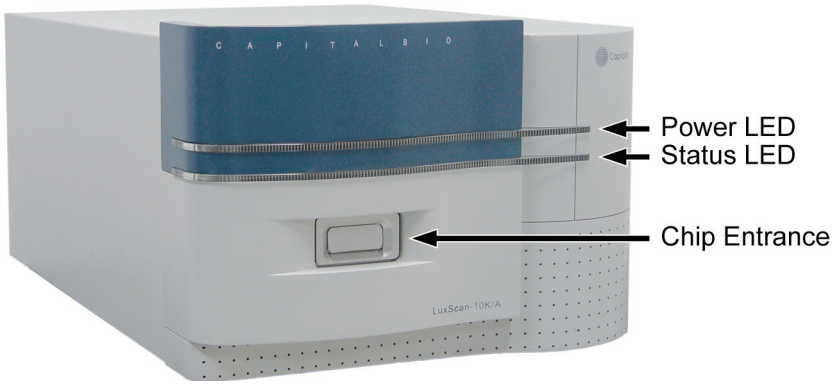
1.3.3 Listing of Items

Check the *Packing List* within the package for all items included in the package of LuxScan™ 10K Microarray Scanner.

Please retain all packing materials. Additional shipping charges will be assessed if packing is unavailable for the system warranty service.

1.4 Hardware Introduction

1.4.1 Front Panel

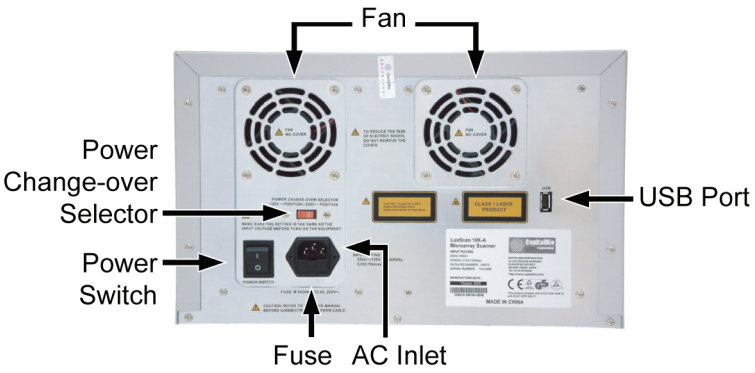


	Note: Do not insert anything except the standard chips defined in this manual. Do not expose your eyes to the inside of *Chip Entrance* when the scanner is working.

LED descriptions:

	LED	Status	Description
1	Power	On	Scanner powered on, but is not connected to the computer workstation.
	Status	Flashing	
2	Power	On	Scanner is ready for scanning.
	Status	Green	
3	Power	On	Scanner is scanning but will not response to any user's command, unless the current scanning is stopped.
	Status	Yellow	
4	Power	On	Errors occurred, please refer to Chapter 6 for "Troubleshooting"
	Status	Red	
5	Power	Off	Scanner is powered off.
	Status	Off	

1.4.2 Rear Panel



	Note: Do not cover or insert anything into the fan windows.

1.4.3 Cables

Power Cable

USB Cable

	Note: Do not use a USB cable longer than 3 meters.

1.5 Installation

Step 1

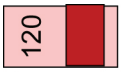
Gently unpack and place the scanner on a level and sturdy bench surface. Both the scanner and computer workstation should be powered off.

Step 2

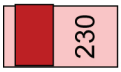
Connect the USB cable, one end to the scanner and the other to the computer workstation.

Step 3

Set the 120V/230V switch according to your power supply, as shown in the following:



120V Power Supply



230V Power Supply

Step 4

Connect the power cable to the scanner.

Note:

To reduce the risk of electric shock, the LuxScan™ 10K scanner must be grounded. The power cable provided with the system provides a grounded connection.

Overall view after installation:

Note:

We recommend that the user should install the "*USB 2.0 PCI Adaptor*" provided with the scanner into the computer workstation to connect with the scanner. The adaptor should be installed by experts or by technical support of the computer workstation provider.

Installation will avoid some USB compatibility issues and improve the scanner performance.

1.6 Power On the LuxScan™ 10K Scanner

Step 1

Power on the computer workstation.

Step 2

Power on the scanner. Make sure that the fans are running, and the power LED and status LED lights are on.

Step 3

If the LuxScan™ 10K application software has been successfully installed on the computer workstation, the operating system will automatically detect and install the driver of the scanner (See next section). Otherwise, you should have to shutdown the scanner, install the software (See Chapter 2 "Software Installation" for details), and restart the scanner.

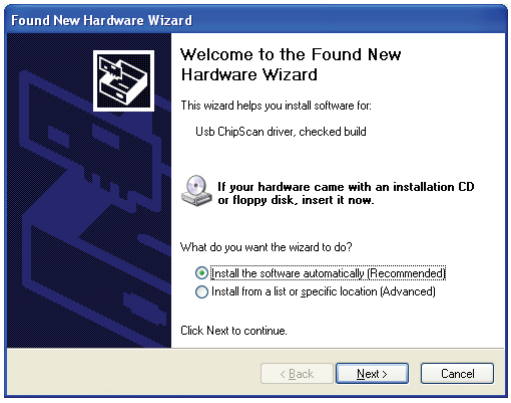
If the scanner is detected by the computer workstation, you will find a USB device icon in the notification area of the Microsoft Windows® taskbar of the computer workstation.

Step 4

Launch the application software, click on the laser control settings to switch on and prepare the lasers for scanning. After approximately 10 minutes, the LuxScan™ 10K scanner will be ready for scanning.

See following chapters for software installation and operation.

1.7 Install Device Driver


Found New Hardware Wizard
Welcome to the Found New Hardware Wizard
This wizard helps you install software for:
Usb ChipScan driver, checked build
If your hardware came with an installation CD or floppy disk, insert it now.
What do you want the wizard to do?
Install the software automatically (Recommended)
Install from a list or specific location (Advanced)
Click Next to continue.
< Back
Next >
Cancel

Step 1:

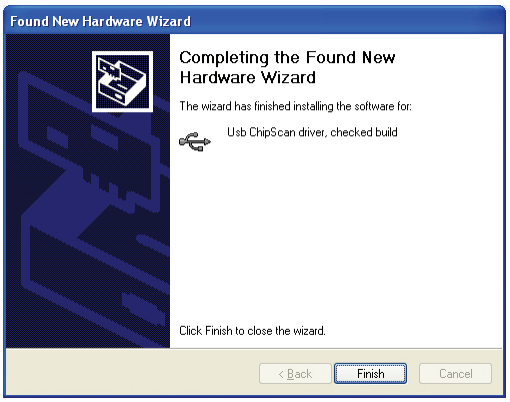
When the scanner is connected to the computer workstation and powered on, the computer workstation will find the new device and load the installation wizard.

Choose "Install the software automatically" and click the **Next** button.

Hardware Installation
The software you are installing for this hardware:
Usb ChipScan driver, checked build
has not passed Windows Logo testing to verify its compatibility with Windows XP. (Tell me why this testing is important.)
Continuing your installation of this software may impair or destabilize the correct operation of your system either immediately or in the future. Microsoft strongly recommends that you stop this installation now and contact the hardware vendor for software that has passed Windows Logo testing.
Continue Anyway
STOP Installation

Step 2:

The computer workstation will find the driver, and prompt for installation. Click the **Continue Anyway** button.


Found New Hardware Wizard
Completing the Found New Hardware Wizard
The wizard has finished installing the software for:
Usb ChipScan driver, checked build
Click Finish to close the wizard.
< Back
Finish
Cancel

Step 3:

After the driver is successfully installed, click the **Finish** button to close the wizard.

1.8 Shutdown the System

Step 1

Save and close all opened images on the screen of the computer workstation.

Step 2

Exit from the LuxScan™ 10K application software.

Step 3

Click the USB device icon on Microsoft Windows® taskbar notification area, and select "Unplug or eject hardware".

Step 4

Power off the scanner.

	Note: After powering off the scanner, you should wait at least 30 seconds before powering it on again.

Chapter 2 Software Installation

2.1 Overview

The LuxScan™ 10K application software and device driver should be installed on the computer workstation which you want to connect to and control the LuxScan™ 10K scanner.

The application software provides the following functions:

- Scanner control, laser and loader control
- Scan protocol management
- Preview scan and scan multiple ROIs
- Image acquisition and visualizations
- Image analysis, block auto-alignment and data acquisition
- Histogram and scatter plot analysis

2.2 Requirements

To install and run LuxScan™ 10K application software, a computer workstation installed with Microsoft Windows® XP (with service pack 2) is recommended. The minimal hardware requirements are listed in section 1.2.5.

When the software is not connected to a LuxScan™ instrument, a *Hardware Protection Key* must be used to enable some functions which include saving images or results and scatter plot analysis.

The software will prompt user to install it when the *Hardware Protection Key* is needed.

2.3 Software Installation

2.3.1 Preparations

Step 1

Power on the computer workstation, login as **Administrator**.

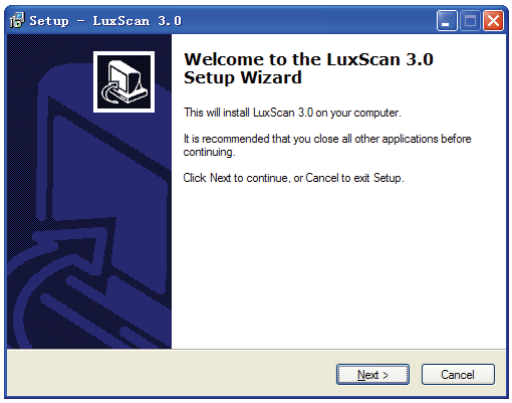
Step 2

Insert the provided LuxScan™ 10K CD into the CD-ROM driver on the computer workstation.

Step 3

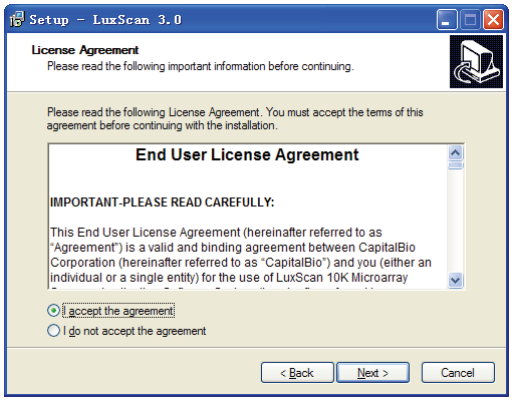
Browse the CD-ROM and find the file named “LuxScan3.0Setup.exe”. Double click this file to launch the installation wizard.

2.3.2 Installation Wizard


Setup - LuxScan 3.0
Welcome to the LuxScan 3.0 Setup Wizard
This will install LuxScan 3.0 on your computer.
It is recommended that you close all other applications before continuing.
Click Next to continue, or Cancel to exit Setup.
Next >
Cancel

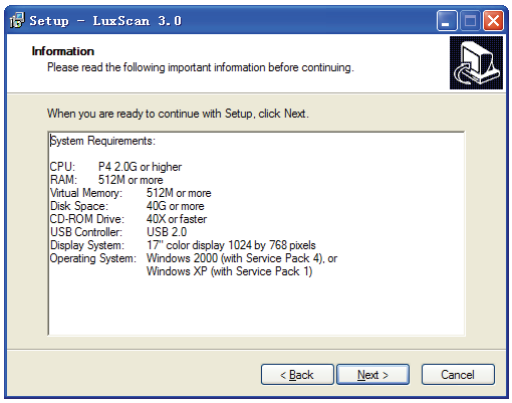
Step 1

Greeting page. Click the **Next** button to proceed.


Setup - LuxScan 3.0
License Agreement
Please read the following important information before continuing.
Please read the following License Agreement. You must accept the terms of this agreement before continuing with the installation.
End User License Agreement
IMPORTANT-PLEASE READ CAREFULLY:
This End User License Agreement (hereinafter referred to as "Agreement") is a valid and binding agreement between CapitalBio Corporation (hereinafter referred to as "CapitalBio") and you (either an individual or a single entity) for the use of LuxScan 10K Microarray
I accept the agreement
I do not accept the agreement
< Back
Next >
Cancel

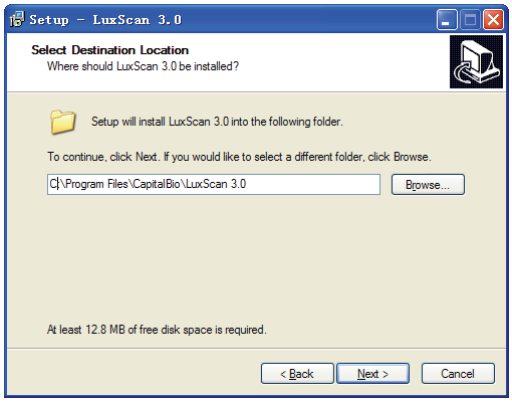
Step 2

License agreement. You should read this agreement carefully. You must choose "*I accept the agreement*" option to continue the installation.


Setup - LuxScan 3.0
Information
Please read the following important information before continuing.
When you are ready to continue with Setup, click Next.
System Requirements:
CPU: P4 2.0G or higher
RAM: 512M or more
Virtual Memory: 512M or more
Disk Space: 40G or more
CD-ROM Drive: 40X or faster
USB Controller: USB 2.0
Display System: 17" color display 1024 by 768 pixels
Operating System: Windows 2000 (with Service Pack 4), or Windows XP (with Service Pack 1)
< Back
Next >
Cancel

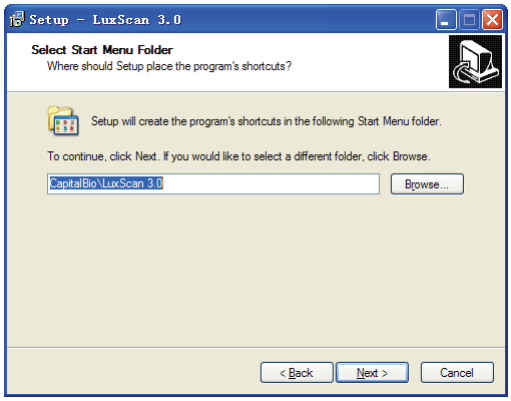
Step 3

System requirements. Check these items and make sure your computer meets all the requirements.


Setup - LuxScan 3.0
Select Destination Location
Where should LuxScan 3.0 be installed?
Setup will install LuxScan 3.0 into the following folder.
To continue, click Next. If you would like to select a different folder, click Browse.
C:\Program Files\CapitalBio\LuxScan 3.0
Browse...
At least 12.8 MB of free disk space is required.
< Back
Next >
Cancel

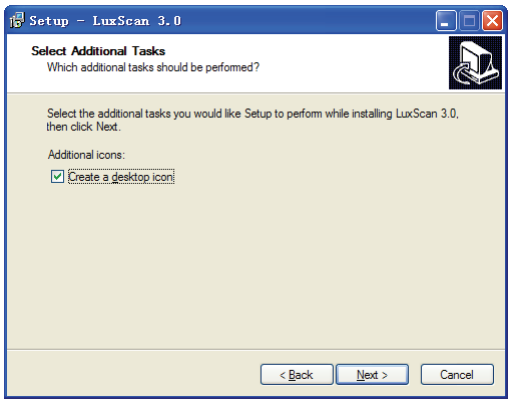
Step 4

Choose a folder to install the software.


Setup - LuxScan 3.0
Select Start Menu Folder
Where should Setup place the program's shortcuts?
Setup will create the program's shortcuts in the following Start Menu folder.
To continue, click Next. If you would like to select a different folder, click Browse.
CapitalBio\LuxScan 3.0
Browse...
< Back
Next >
Cancel

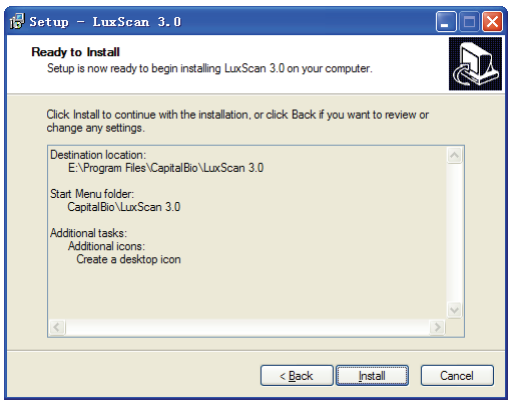
Step 5

Choose a program group which will be installed into the **Start** menu of the Microsoft Windows® desktop.


Setup - LuxScan 3.0
Select Additional Tasks
Which additional tasks should be performed?
Select the additional tasks you would like Setup to perform while installing LuxScan 3.0, then click Next.
Additional icons:
Create a desktop icon
< Back
Next >
Cancel

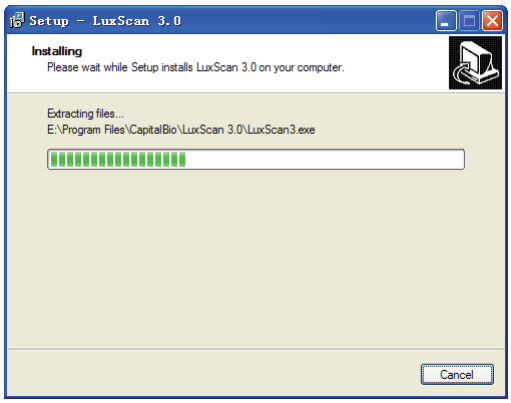
Step 6

Choose whether a desktop shortcut should be created.


Setup - LuxScan 3.0
Ready to Install
Setup is now ready to begin installing LuxScan 3.0 on your computer.
Click Install to continue with the installation, or click Back if you want to review or change any settings.
Destination location:
E:\Program Files\CapitalBio\LuxScan 3.0
Start Menu folder:
CapitalBio\LuxScan 3.0
Additional tasks:
Additional icons:
Create a desktop icon
< Back
Install
Cancel

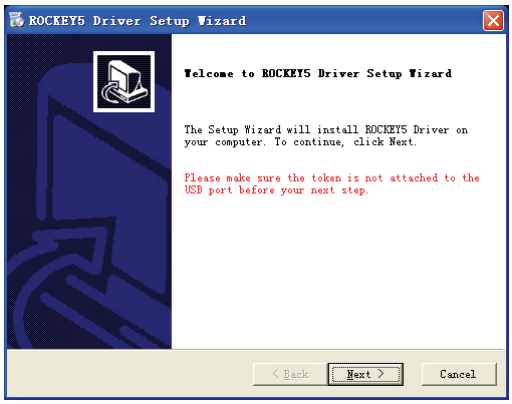
Step 7

Check the installation settings. The installation will proceed once you click the **Install** button.



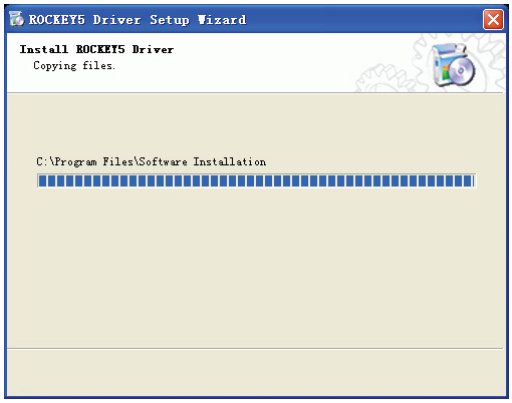
Step 8

An animated status bar shows the installation process.


ROCKEY5 Driver Setup Wizard
Welcome to ROCKEY5 Driver Setup Wizard
The Setup Wizard will install ROCKEY5 Driver on your computer. To continue, click Next.
Please make sure the token is not attached to the USB port before your next step.
< Back
Next >
Cancel

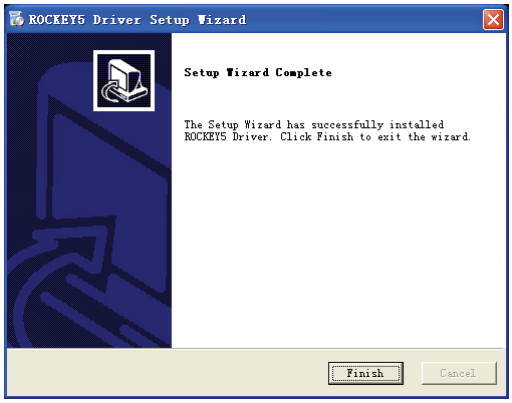
Step 9

Greeting page for *Hardware Protection Key* driver installation. Click the **Next** button to proceed.



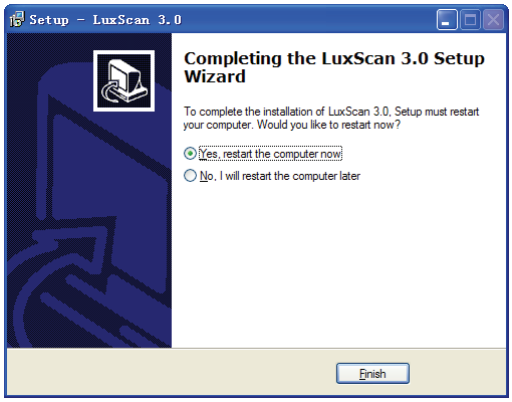
Step 10

A status bar indicates the progress of the *Hardware Protection Key* driver installation process.


ROCKEY5 Driver Setup Wizard
Setup Wizard Complete
The Setup Wizard has successfully installed ROCKEY5 Driver. Click Finish to exit the wizard.
Finish
Cancel

Step 11

Completion of *Hardware Protection Key* driver installation. Click the **Finish** button to proceed.


Setup - LuxScan 3.0
Completing the LuxScan 3.0 Setup Wizard
To complete the installation of LuxScan 3.0, Setup must restart your computer. Would you like to restart now?
Yes, restart the computer now
No, I will restart the computer later
Finish

Step 12

Installation succeeded. Click the **Finish** button to exit. The system will restart to update the changes, or you can choose to restart the computer workstation later.

Note:

During the installation, you can click the **Back** button to modify previous settings and you can click the **Cancel** button to exit the installation.

2.4 Uninstall

Like most Microsoft Windows® applications, you can uninstall the software from *Windows Control Panel*. Launch "Add/Remove Programs" item in the *Windows Control Panel*; find "LuxScan 3.0" and choose **Change/Remove**; when a confirmation is prompted, choose **Yes**, then the software will be removed from the computer workstation.

Chapter 3 Chip Scanning Tutorial

3.1 Overview

In this chapter, we will guide you to scan a microarray chip. This way, you can quickly grasp the basic skills to use the software and accomplish basic scanning tasks.

Whenever a new system is installed, or the system is moved to a new place, or some changes are made to the hardware or the software, we recommend you to scan the calibration chip to ensure that the system functions normally.

Note:

LuxScan™ software provides two separate application functions:

- Microarray scan and data acquisition
- Scan data analysis

The LuxScanTM 10K-A microarray scanner acquires scanning data from each channel sequentially. It does not scan microarrays using both channels simultaneously. Sequential scanning of channels eliminates cross-talk.

Compatible dyes are listed below:

Red laser channel (635nm): Cy5, BODIPY 630/650, Alexa 633, Alexa 647, BODIPY 650/665, Alexa 660, Cy5.5, Alexa 680.

Green laser channel (532nm): Cy3, Alexa 532, POPO-3, PO-PRO-3, Alexa 546, Alexa 555, Alexa 568, Cy3.5.

3.2 Launch the System

3.2.1 Start Up

Step 1 Power on the computer workstation.

Step 2 Connect the scanner and the computer workstation with the USB cable and power on the scanner. The USB device icon should appear in the Microsoft Windows® taskbar.

Step 3 Start the LuxScan™ 10K application software by clicking **Programs > CapitalBio > LuxScan 3.0 > LuxScan 3.0** from the **Start** menu. It will search for the scanner and perform self testing.

3.2.2 Connect to Scanner Device

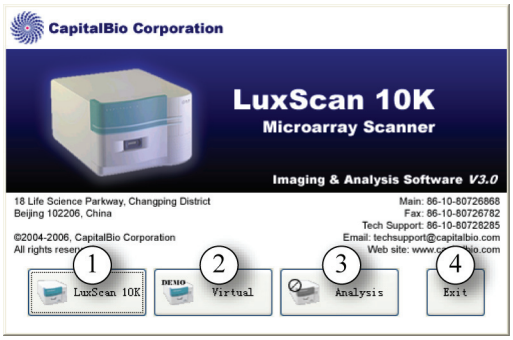


Fig 3.1 Splash Window

In the splash window, select one of working modes:

1. Connect to the **LuxScan™** scanner instrument.
2. Connect to a software simulated **Virtual** scanner instrument.
3. Analyze previously scanned images without connecting to any scanner instrument.
4. Exit the application.

Note:

The **Virtual** scanner is used for software evaluation and offline training. Users can use this mode to try and learn the software safely.

3.3 Interface Descriptions

3.3.1 Main window

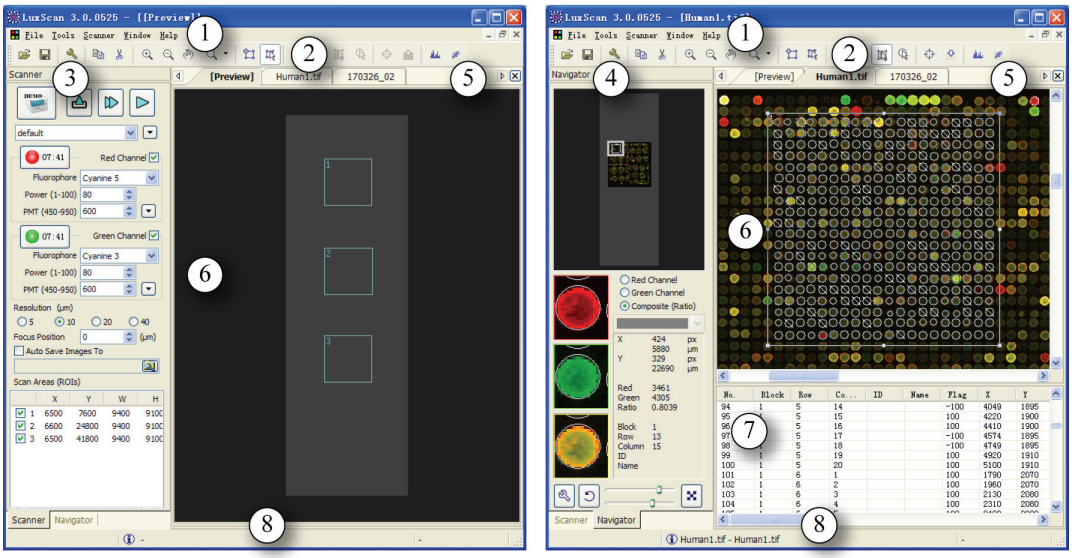


Fig 3.2 Scan and Image Analysis Interfaces

Item	Panel	Description
1	*Menu Bar*	Commands and controls.
2	*Tool Bar*	Commands and controls.
3	*Scanner Panel*	Control scanner, setup scan parameters.
4	*Navigator Panel*	Overview of current working image and setup visual effects.
5	*Document Tab*	Switch between working documents.
6	*Image Window*	Display working images, scan areas and grids.
7	*Data Window*	Display image analysis result data.
8	*Status Bar*	Display information and scan progress.

3.3.2 Tool Bar

Fig 3.3 Tool Bar

Item	Command	Description
1	**Open File**	Open scan images for analysis.
2	**Save File**	Save scan images and analysis results.
3	**Settings**	Image analysis and miscellaneous options.
4	**Copy**	Create a copy of selected scan area or *block*.
5	**Delete**	Delete selected scan area or *block*.
6	**Zoom In**	Enable zoom in control mode.
7	**Zoom Out**	Enable zoom out control mode.
8	**Move**	Enable move control mode.
9	**Zoom to Fit**	Fit image in the window.
10	**Create *ROI***	Create a new scan area (*ROI*).
11	***ROI* Mode**	Enable *ROI* control mode.
12	**Load Array List File**	Load blocks from an Array List (GAL or XAL) file.
13	**Create *Block***	Create new *blocks*.
14	***Block* Mode**	Enable *block* control mode.
15	***Circle* Mode**	Enable *circle* control mode.
16	**Align *Blocks***	Auto-align blocks to image.
17	**Extract Data**	Acquire *spot* data from image.
18	**Histogram**	Enable histogram analysis tool.
19	**Scatter Plot**	Enable scatter plot analysis tool.

3.3.3 Side Panels

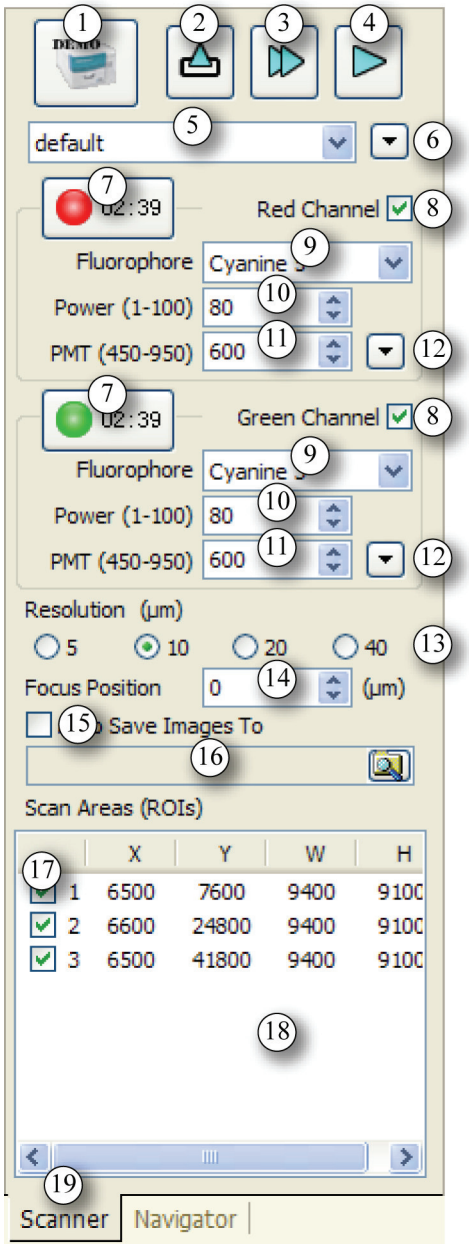


Fig 3.4 Scanner Panel

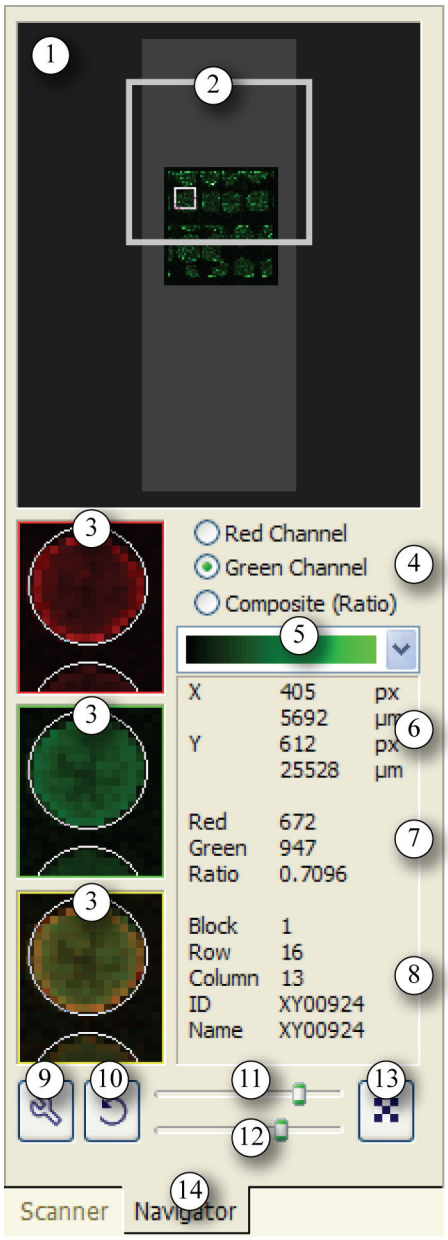


Fig 3.5 Navigator Panel

See following sections for detailed descriptions.

3.3.4 Scanner Panel

Item	Name	Description
1	*Scanner Info*	Display scanner type and information.
2	*Loader Button*	Eject and reset scanner chip loader.
3	*Preview Scan Button*	Start Preview Scan.
4	*Scan Button*	Start Scan.
5	*Protocol List*	Select current scan protocol.
6	*Protocol Menu Button*	Manage protocols.
7	*Laser Buttons*	Control ON/OFF of laser and display warming timer.

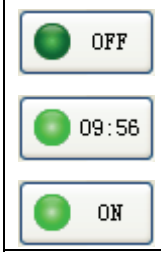
Item	Name	Description
8	*Channel Controls*	Enable/Disable the channel.
9	*Fluorophore List*	Select fluorophore type.
10	*Laser Power*	Setup Laser Power parameter.
11	*PMT Gain*	Setup PMT Gain parameter.
12	*PMT Amplifier Menu*	Setup PMT amplification ratio.
13	*Resolutions*	Setup the scan resolution.
14	*Focus Position*	Setup the scan focus range.
15	*Auto Save*	Enable/Disable auto-save function.
16	*Auto Save Folder*	Select a folder to auto save images into.
17	*Scan Area Control*	Enable/Disable a scan area (*ROI*).
18	*Scan Area List*	Information of scan areas (*ROI*).
19	*Side Panel Tab*	Switch to scanner panel.

3.3.5 Navigator Panel

Item	Name	Description
1	*Navigator Window*	Control the visible region of the image window, click or drag the yellow rectangle will change the visible part of current image window.
2	*View Box*	Visible region of image view.
3	*Local Viewer*	A 4:1 view of local part of the image according to current cursor position or selected spot.
4	*Channel Selector*	Set visible channel: Red, Green or Composite.
5	*Color Palette Selector*	Select color palette for current channel.
6	*Cursor Information*	Display position of mouse cursor.
7	*Pixel Information*	Display current pixel intensities and ratio.
8	*Spot Information*	Display current spot information.
9	*Settings Button*	Setup image color mapping settings.
10	*Reset Button*	Reset brightness and contrast.
11	*Slide Brightness Control*	Tune visual brightness of current image window.
12	*Slide Contrast Control*	Tune visual contrast of current image window.
13	*Auto Brightness and Contrast*	Auto-find best brightness and contrast settings.
14	*Side Panel Tab*	Switch to navigator panel.

3.4 Preparation

3.4.1 Switch on Lasers

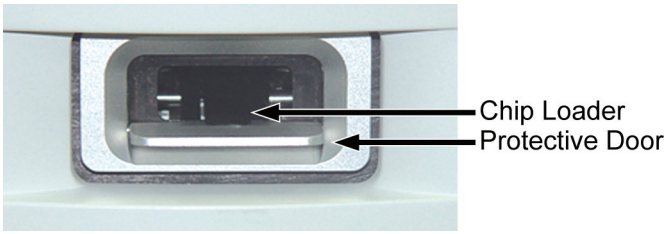


Click the **Laser** button on the *Scanner Panel*. The button icon will change from OFF to ON.

A clock counting down 10 minutes shows the warming-up process of the laser.

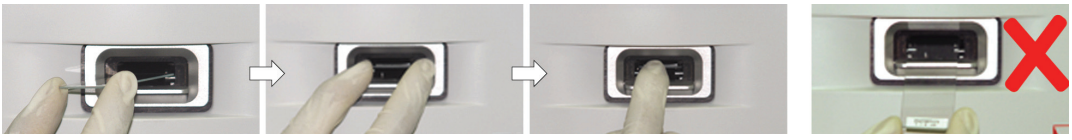
3.4.2 Load a Chip into Scanner

While waiting for the warm-up to complete, the user can prepare the chip.



	Click the menu **Scanner** and select item **Eject Loader**, or click the **Eject Loader** button on the *Scanner Panel*. The loader platform to hold the chip will emerge from the loading slot entrance.

Place the chip squarely onto the loader platform and push the chip slowly and gently back into the slot entrance horizontally. Push the chip to the end, until it is locked into the loader.



	Note: The chip must be inserted into the scanner with the spotted side up. Make sure the loader platform has emerged fully from the slot entrance. Ensure that the chip is seated correctly on the loader platform. Inserting the chip obliquely may damage the scanner and the chip.

	Click the **Reset Loader** button again to reset the loader and the chip will be loaded automatically into the scanner.

3.4.3 Select Channels and Setup Scan Parameters

Click the **Red Channel** or **Green Channel** check boxes on the *Scanner Panel* to enable/disable the channel to scan. If you enable both of them, the red channel will come first and then the green channel.

Setup the scan parameters for each channel. The default scan parameters are recommended for initial uses. See Chapter 4 for detailed parameters descriptions.

3.4.4 Preview Scan

Preview scan is a quick scan mode which is used to overview the whole microarray. The scan resolution for preview scan is fixed to 40 μm, and the area of the preview scan is the maximum scan area.

	Click the **Preview Scan** button to start the scan process. The *Scan Progress Bar* at the bottom of the window shows the percentage of the work finished.

After the preview scan, an image will be displayed in the [Preview] tab of the image window.

See Chapter 4 for detailed controls on the image windows.

3.5 Scan the Chip

3.5.1 Create the Scan Areas (ROIs)

	Click the **Add Scan Area** button on the *Tool Bar*, drag a rectangle on the image window and a *Scan Area (ROI)* will be created
	Make sure the image window is switched to *ROI* control mode.

Dragging the rectangle or the corner handles will change its position and size. The position of the scan area will be displayed in the *Scan Area List* at the bottom of the *Scanner Panel*. Double click a selected scan area will **Enable** or **Disable** it in the next scan.

Multiple *ROIs* can be created to be scanned sequentially.

3.5.2 Select Channels and Setup Scan Parameters

Click the **Red Channel** or **Green Channel** check boxes on the *Scanner Panel* to enable/disable the scan of the channel. Images scanned in the dual channels will be layered into the same image window to generate a *Composite Image (Ratio Image)*.

Setup the scan parameters for each channel. The default scan parameters are recommended for initial uses. See Chapter 4 for detailed parameters descriptions.

3.5.3 Start Scan

	Click the **Start Scan** button to start the scan.
	The **Stop Scan** button can be used to stop the current scan.

3.5.4 Save Scan Images

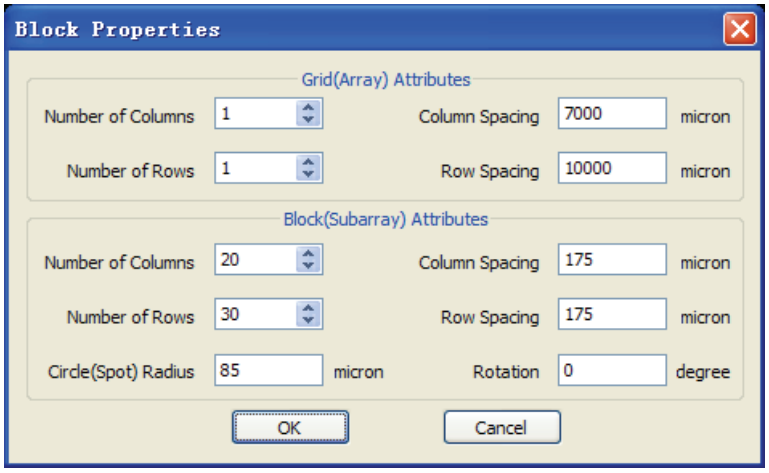
	Click the **Save** button. In the *Save Files* dialog box, input a file name and select file formats to save.

The scan image **MUST** be saved in TIFF format for later analysis. The color images in JPEG/BMP/PNG format can also be saved, but they are for visualization purpose only and can not be used for the analysis of microarray data.

If the **Composite** option is selected, images in dual channels will be saved into a TIFF file of two separate pages, and the color image will be a composite image.

3.6 Image Analysis

3.6.1 Create Blocks



	Click the **Add *Blocks*** button on the *Tool Bar* to create a set of *blocks* on the image. In the *Block Properties* dialog box, setup the number of *circle* rows and columns, spacing of *circle* rows and columns, *circle* radius and rotation of the *block*. Multiple *blocks* can be created by setting-up the rows and columns of the *blocks* and the spacing of the rows and columns.

3.6.2 Load Array List File

	If an Array List (GAL or XAL) file exists for the microarray, *blocks* with feature ID and Names can be loaded from it. Click the **Load Array** button on the *Tool Bar* and select the array list file.

3.6.3 Manual Align Blocks to Spots

	Make sure the image window is switched to *Block* control mode.

Before auto-aligning the *blocks* to the *spots*, the *blocks* and *circles* must be put close to the *spots* manually. Use the mouse cursor to drag the *block* and the handles to modify its position, size, shape and rotation. See Chapter 4 for detailed mouse controls in *Block Mode*.

3.6.4 Auto Align Blocks

	Click the **Align All** button on the *Tool Bar* or press **F6** key to align all *blocks* on the image, or to align selected *blocks* only press **F5**.

After alignment, each *circle* will be fitted to a *spot*. *Circles* that failed to align will be marked by back slashes.

3.6.5 Acquire Data

	Click the **Extract Data** button on the *Tool Bar* or press **F7** button to acquire signal and background data from current images. The data will be displayed in the table window below the image window.

	See Chapter 4 for detailed descriptions of each data column.

3.6.6 Save Data

	Click the **Save** button on the *Tool Bar* and select the **LSR** checkbox on the *Save Files* dialog box.

LuxScan 3.0 saves the data into LuxScan Result File (LSR) format, which can then be opened by most spread-sheet software (e.g. Microsoft Excel).

3.7 Remove the Chip and Exit

	Click the **Eject Loader** button to eject the chip loader and remove the chip.
	After the chip is removed, click the **Reset Loader** button to withdraw the chip loader.

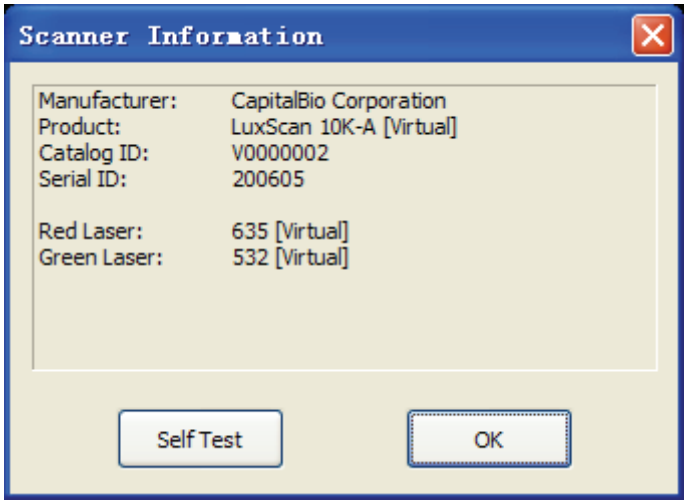
Switch off the lasers and close the *Main Window*.

Chapter 4 Additional Software Features

4.1 Overview

This chapter describes more features of the LuxScan™ 10K application software, including system self-testing, image visualization controls, scan parameters, image composition and file manipulation, etc.

4.2 Scanner Information and Self Testing



	Click the **Scanner Info** button on the *Scanner Panel*, or click the **Scanner** menu and select **Scanner Info** to view the scanner information.

Click the **Self Test** button to start the scanner self test.

You can use this feature to inspect whether the LuxScan™ 10K system functions normally, or to reset the system to its original state.

	Note: If the scan or self test failed, please restart the scanner and software by: 1. Power off the scanner instrument. 2. Save your work and exit the application software. 3. Restart scanner after at least 30 seconds down-time and then restart the software.

4.3 Image Window Controls

4.3.1 Control Modes

	Mode	Shortcut Key	Description
	Zoom In	**Z**	Enlarge the image in *Image Window* by dragging a region or click the left mouse button.
	Zoom Out	**X**	Shrink the image in *Image Window* by dragging a region or click the left mouse button.

	Move	**V**	Move visible part of the *Image Window* by dragging the mouse cursor.
	ROI Mode	**R**	Control *Scan Areas (ROIs)* on the *Image Window*.
	Block Mode	**B**	Control *Blocks* on the *Image Window*.
	Circle Mode	**C**	Control *Circles* on the *Image Window*.

4.3.2 Commands

	Command	Shortcut Key	Description
	Zoom to Fit	**F3**	Fit the image to the *Image Window*.
	Fit Object	**F4**	Fit the select scan areas or *blocks* to the *Image Window*.
	Copy	**Insert**	Clone current selected *ROIs* or *blocks*.
	Delete	**Delete**	Delete current selected *ROIs* or *blocks*.

4.3.3 Mouse Controls

Action	Description
Left Click	Select a *ROI*, *block* or *circle*.
Drag a Region	Select all objects in the dragged region.
Shift Key + Select	Add selected objects to the current selection.
Left Double Click	Enable/disable selected *ROIs*, or set *block* properties.
Drag Selected Objects	Move selected objects.
Drag Corner Handle	Resize *ROI* or *block*.
Drag Middle Handle	Rotate a *block*.
Right Click while Dragging	Cancel current movement.
Right Click Selected Object	Pop out context menu.
Control Key + Wheel	Zoom in/out the image view.
Right Down + Wheel	Zoom in/out the image view.
Middle Down + Wheel	Move image view.
Control Key + Drag a Region	Create a *ROI* in *ROI* mode.
Control Key + Drag a *Block* Corner Handle	Change the *block* frame to an irregular quadrilateral.
Wheel in Selected *Circle*	Change *circle* size.

4.4 Visual Effects

The pixels in each channel of a scan image are 16-bit gray scale signals. The software provides several methods to view the image in different effects for visual analysis. The controls are provided on the *Navigator Panel* (see "3.3 Side Panels"). No matter how the visual effects are set, the signals in the scan image are never modified.

4.4.1 Select Channel and Color Palette

Each channel can be shown individually with different color palette by using control 4 and 5 in Fig 3.5. While dual channels are visible in composite mode, their default color palettes are used.

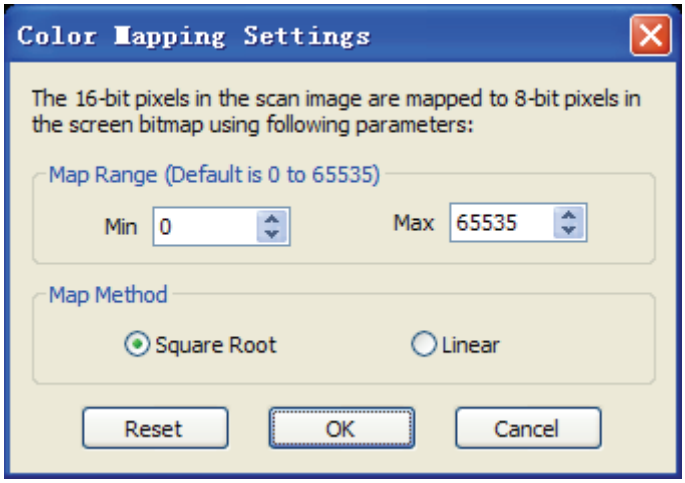
4.4.2 Brightness and Contrast

The user can tune the brightness and contrast of the image by using the slide controls at the bottom of the *Navigator Panel*.

	Click the **Auto** button to adjust the brightness and contrast automatically.
	Click the **Reset** button to reset the brightness and contrast.

4.4.3 Color Mapping

	Click the **Settings** button to set the color mapping settings.

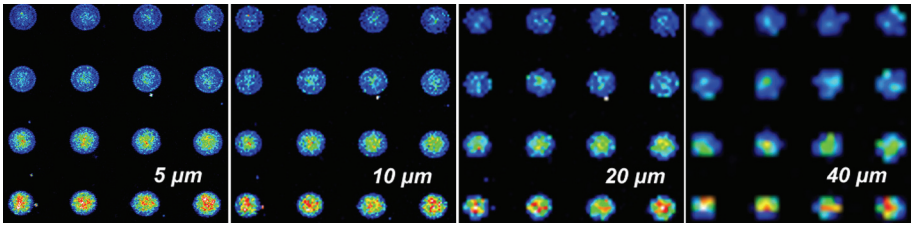


The *Map Range* is the range of pixel signals in a scan image which is to be mapped to colors on the screen. Signals less than the range are mapped to 0, and signals greater than the range are mapped to 255.

The *Map Method* is the math method used in mapping signals to pixels. The *Square Root* method is better than the *Linear* method for lower signals. The user can set the default method in the global *Options* dialog box by clicking the **Settings** button on the *Tool Bar*.

4.5 Scan Parameters

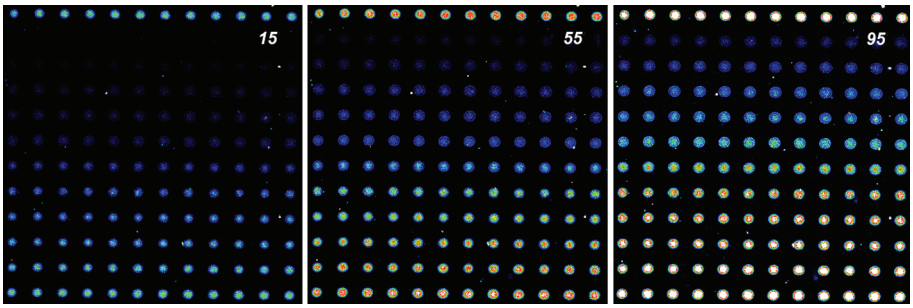
4.5.1 Resolution



Increasing the resolution can improve the definition of the image pixels, but it also requires a longer scanning time and results in a larger image. Usually, when you adjust the parameters, or search for target areas, we recommend selecting low resolution for quick scans. Once the parameters are determined, you can use high resolution to obtain high quality images.

	Note: Scanning one channel on the whole microarray at 5μm resolution will consume about 200Mbyte of memory. Please increase the system memory to optimize the performance of the software. Please save and close scanned images to improve the performance.

4.5.2 Laser Power



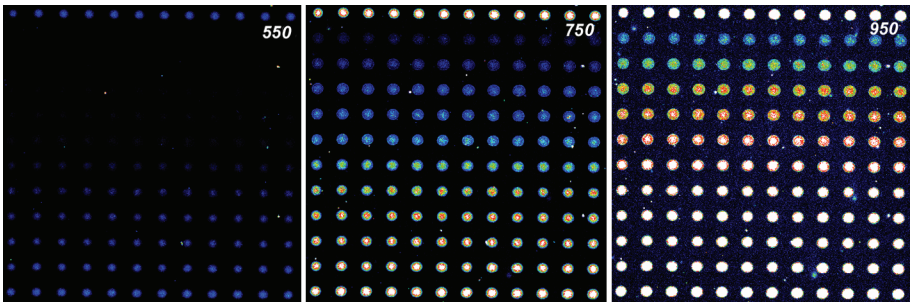
As the laser is the energy source of the fluorescence, the higher the *Laser Power*, the more fluorescence photons the scanner will detect. Increasing *Laser Power* will increase the intensity of image pixels.

The range of *Laser Power* is from 1% to 100%.

Signal intensities will be enhanced when *Laser Power* is increased. The advantage for users to increase *Laser Power* to enhance the signal intensities is that both the background signal intensities and standard deviation of background signal intensities are amplified less than if the signals are increased by increasing the *PMT Gain*.

The *Laser Power* value 95% is the default settings of the scanner. The scanner has been designed and manufactured so that users can get the most valuable data from the chips based on this value for both 532nm laser and 635nm laser. New users or users who require a minimal level of details of the scanner can use this setting during the initial period of usage.

4.5.3 PMT Gain



The Photomultiplier Tube (PMT) refers to the efficiency of transforming a photon signal to an electrical signal. You can increase the *PMT Gain* value for higher signal intensity, but this will also increase the background signal intensity.

The range of *PMT Gain* value is from 450 to 950.

The default setting for *PMT Gain* is 650, whether 635nm laser or 532nm laser is used. At the *PMT Gain* value of 650, users can get the best dynamic range and detection limits from the scanner. It is also recommended for new users and users who require a minimal level of detail from the scanner to apply this setting.

When the sample concentration is already fixed for certain experiments, *PMT Gain* may be increased or decreased from the default value of 650 to get the required signal intensities.

The *PMT Amplification Menu* (control 12 in Fig 3.4) can be used to adjust the PMT according to signal changes. For example, select ×2.00 will set the PMT to double the signal intensities.

Note:

It is critical for a user to set up proper *Laser Power* and *PMT Gain* parameters to achieve the optimal scanning quality. Different microarrays may have different optimal working parameters and the user may need to try a few testing scans to determine them.

The behavior of the *Laser Power* and *PMT Gain* parameters for LuxScan™ 10K Microarray Scanner are different from those of other manufacturers' scanners. This difference in the parameter settings has no effect on performance factors.
You may need to setup different *Laser Power* and *PMT Gain* parameters to balance the dual channel signal on the same chip. See section 4.8 for details.

4.5.4 Focus Position

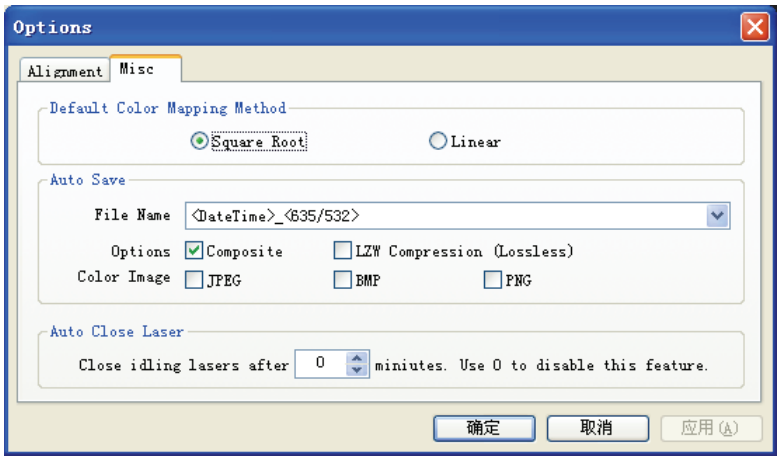
By default, the *Focus Position* of the scanner is positioned on the top surface of the chip. Users can set *Focus Position* manually.

The range of *Focus Position* is from -250μm to +250μm.

4.5.5 Auto Save

If the **Auto Save** option is enabled in the *Scanner Panel*, each scan image will be saved to selected directory automatically.

The user can select a method to name the image and select the formats of color images to be saved with the TIFF format scan image, by clicking the **Settings** button on the *Tool Bar* in the *Options* dialog box.



4.5.6 Protocol Management

A set of scan parameters can be stored to a *Protocol* for future scans. *Protocol* controls are available on the top of *Scanner Panel* (*see* Controls 5 and 6 *in* Fig 3.4, *page 28 of Manual*). Click the button beside the *Protocol* name to get more commands.

Command	Description
New	Create a new protocol.
Save	Save current protocol.
Save a Copy As	Save current protocol to a copy.
Reset	Reset protocol to last saved status.

Command	Description
Delete	Delete current protocol.

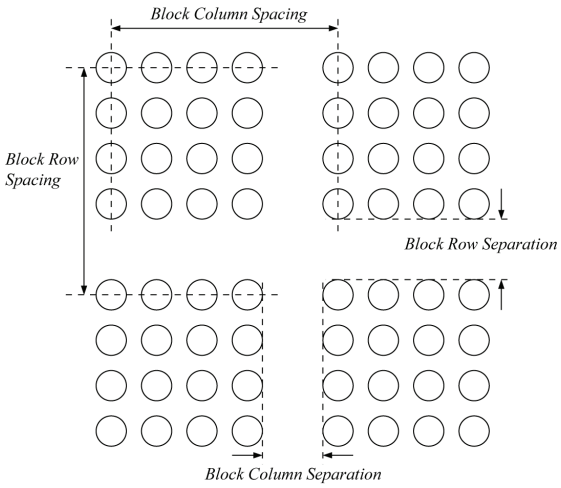
4.6 Image Data Analysis Principles

4.6.1 Basic Concepts

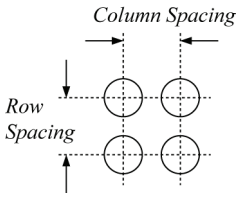
On Microarray	Tools For Image Analysis	Definition
Spot	*Circle*	*Circle*s are the circlets generated by the software to visually define the boundaries of the *Spot*s on the image being analyzed. All the pixels inside a *circle* are treated as *spot* intensity. The pixels outside the *circle* region are used to calculate the *spot* background intensity except the pixels adjacent to the *circle* outside perimeter.
Subarray	*Block*	A *block* is a collection of *circles* drawn on an image. Each *block* corresponds with the *subarray* on the *array* chip or the *array* image.
Array	*Grid*	The collection of all *blocks* of a microarray chip is called a *grid*. *Circle*s of a *grid* correspond with all the arrayed *spot*s on the microarray chip.

Grid scope concepts:

Term	Definition
Block Column Spacing	*Block Column Spacing* is the distance, in microns, between the first column *circle* centers of adjacent *block*s.
Block Row Spacing	*Block Row Spacing* is the distance, in microns, between the first row *circle* centers of adjacent *block*s.
Block Column Separation	*Block Column Separation* is the distance, in microns, between the edges of adjacent *block*s in the same column.
Block Row Separation	*Block Row Separation* is the distance, in microns, between the edges of adjacent *block*s in the same row.
Rotation	*Rotation*, in degrees, is the angular degree of rotating the *block*s anti-clockwise. A negative value means rotating the *block*s in the clockwise direction.



Grid Scope Concepts

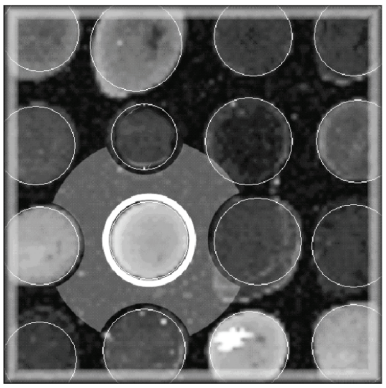


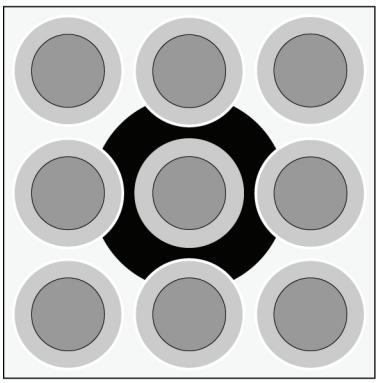
Block Scope Concepts

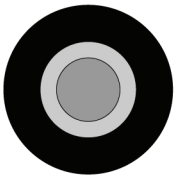
Block scope concepts:

Term	Definition
Column Spacing	*Column Spacing* is the distance, in microns, between the centers of adjacent *circle*s in the same row.
Row Spacing	*Row Spacing* is the distance, in microns, between the centers of adjacent *circle*s in the same column.

4.6.2 Background Definitions







Background region

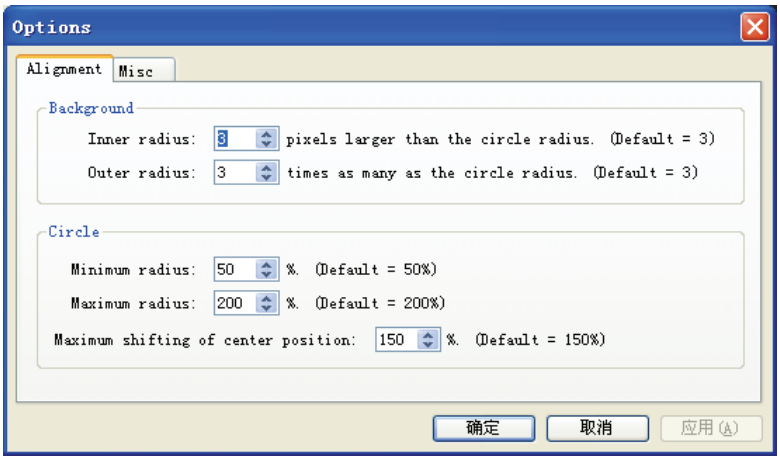
Neutral Zone: a (1-8 pixels wide) zone outside the *spot* region

Spot region

Background is a user definable region surrounding the *circle*, which

- Can have a radius 2-4 times of the circle radius,
- Excludes all the pixels inside the *circle* or its adjacent *circles,* and
- Excludes the 1-8 pixels of the *Neutral Zone* surrounding these *circles*.

	Click the **Settings** button to pop out *Options* dialog box and change the settings for image analysis.


Options
Alignment
Misc
Background
Inner radius: 3 pixels larger than the circle radius. (Default = 3)
Outer radius: 3 times as many as the circle radius. (Default = 3)
Circle
Minimum radius: 50 %. (Default = 50%)
Maximum radius: 200 %. (Default = 200%)
Maximum shifting of center position: 150 %. (Default = 150%)
确定
取消
应用(A)

4.6.3 Algorithm

Signal

The *spot Signal* is defined as the signal intensities of the pixels within the associated *circle*, and is expressed in terms of *Mean of Intensities*, *Median of Intensities* and *Standard Deviation of Intensities*. A *circle* may be larger or smaller than its associated *spot*, thus the actual number of the pixels of the *spot* can be less or more than the pixels within the *circle*.

Mean of Signal Intensities

The arithmetic mean of intensities of all the pixels within the *circle* is defined as

$$Mean = \frac{\sum I(i,j)}{N}$$

Where *I(i, j)* denotes the intensity *I* of the pixel at row *i* and column *j*; *N* denotes the total number of the pixels within the *circle*.

Median of Signal Intensities

After the intensities of all the pixels within the *circle* are sorted, the pixel value at the middle of the sorted pixels is taken as the *Median of the Signal Intensities*.

Standard Deviation of Signal Intensities

The *Standard Deviation (SD)* of all the pixel values within the *circle* is defined as

$$SD = \sqrt{\frac{\sum (I(i,j) - Mean)^2}{N}}$$

Where *I(i, j)* denotes the intensity *I* of the pixel at row *i* and column *j*; *N* denotes the total number of the pixels within the *circle*, *Mean* is the *Mean of Signal Intensities*.

Background

The *Background* is defined as the pixel intensities outside the associated *circle*. The background pixel intensity can be contributed by auto-fluorescence from microarray slide, fluorescent residues from the hybridization materials and by airborne dusts. The software performs a background extraction to subtract the background noise from the signal.

Mean of Background Intensities

The arithmetic mean of intensities of all the pixels within the *Background* is

$$Mean = \frac{\sum I(i,j)}{N}$$

Where *I(i, j)* denotes the intensity *I* of the pixel at row *i* and column *j*; *N* denotes the total number of the pixels within *Background*.

Median of Background Intensities

After the intensities of all the pixels within the *Background* are sorted, the pixel value at the middle of the sorted pixels is taken as the *Median of Background Intensities*.

Standard Deviation of Background Intensities

The *Standard Deviation* of all the pixel values within the *Background* is defined as

$$SD = \sqrt{\frac{\sum (I(i,j) - Mean)^2}{N}}$$

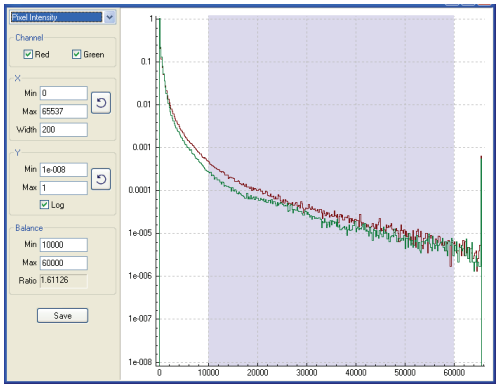
Where *I(i, j)* denotes the intensity *I* of the pixel at row *i* and column *j*; *N* denotes the total number of the pixels within the *Background*; *Mean* is the *Mean of Background Intensities*.

4.6.4 Data Column Definition

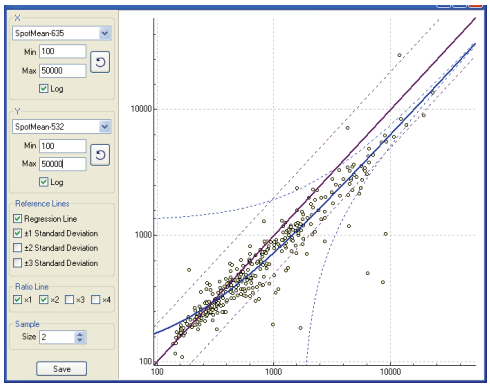
Column Name	Definition
No.	The index number of a *circle* on the *grid*.
Block	The index number of a *block*.
Row	The row number of a *circle*.
Column	The column number of a *circle*.
ID	The accession number of the substance associated with a *circle* derived from an *Array List* file.
Name	The substance name associated with a *circle* derived from an *Array List* file.
Flag	Flag for alignment, positive value means successful alignment and negative value means failed alignment.
Radius	The radius of a *circle*.
X-(#1/#2)	The X-coordinate (in μm) of the center of the *circle* associated with the *spot*, where (0,0) is the top left corner of the image of channel #1 or #2.
Y-(#1/#2)	The Y-coordinate (in μm) of the center of the *circle* associated with the *spot*, where (0,0) is the top left corner of the image of channel #1 or #2.
SpotMedian-(#1/#2)	The median of the values of all the pixels in a *spot* region of channel #1 or #2 image.
SpotMean-(#1/#2)	The mean of the values of all the pixels in a *spot* region of channel #1 or #2 image.
SpotSD-(#1/#2)	The Standard Deviation of the all pixel values in a *spot* region of channel #1 or #2 image.
BkgMedian-(#1/#2)	The median of the values of all the local *background* pixels of channel #1 or #2 image.
BkgMean-(#1/#2)	The mean of the values of all the local *background* pixels of channel #1 or #2 image.
BkgSD-(#1/#2)	The Standard Deviation of the values of all the local *background* pixels of channel #1 or #2 image.
BkgCV-(#1/#2)	The Coefficient of Variance of the values of the local *background* pixels (for the *Scatter Plot* axes only) of channel #1 or #2 image.
SpotCV-(#1/#2)	The Coefficient of Variance of the values of all the pixels in the *spot* region (for the *Scatter Plot* axes only) of channel #1 or #2 image.

Column Name	Definition
S-B_Median-(#1/#2)	The background-subtracted spot median of channel #1 or #2 image.
S-B_Mean-(#1/#2)	The background-subtracted spot mean of channel #1 or #2 image.
Ratio of Medians-(#1/#2)	The ratio of the background-subtracted median intensities of channel #1 to the background-subtracted median intensities of channel #2.
Ratio of Means-(#1/#2)	The ratio of the background-subtracted mean intensities of channel #1 to the background-subtracted mean intensities of channel #2.
Log Ratio of Medians-(#1/#2)	A base ten logarithm of the *Ratio of Medians (#1/#2)*.
Log Ratio of Means-(#1/#2)	A base ten logarithm of the *Ratio of Means (#1/#2)*.

4.7 Histogram and Scatter Plot



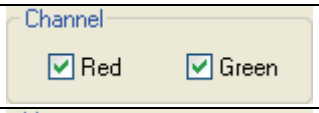
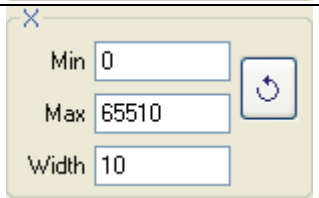
Histogram

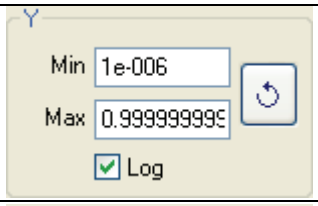
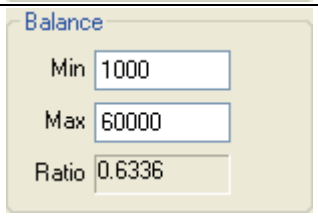
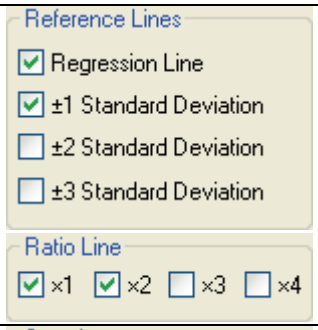
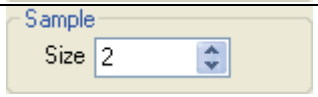
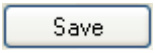


Scatter Plot

	Click the **Histogram** button on the *Tool Bar* to launch *Histogram Window*.
	Click the **Scatter Plot** button on the *Tool Bar* to launch *Scatter Plot Window*.

Controls on the *Histogram* and *Scatter Plot* dialog boxes allow the user to:

	Select the source of data to be displayed in the histogram or for each axis in the scatter plot.
	Set the visibility of each channel in the histogram.
	Set the axis **Min** and **Max** range. Click the **Reset** button to reset the **Min** and **Max** values to full scale. Click **Log** to set the axis to logarithmic scale.

	
	In the histogram, the user can select a range to view the **Ratio** of the sum of signals in each channel, which gives a signal level diversity between the dual channels. The user can use the information to find optimum scan parameters. See *Section* 4.8 for more information.
	Reference lines in the scatter plot.
	Set the diameter of each sample in the scatter plot.
	Click the **Save** button to save the plot to an image file.

4.8 Channel Balancing

The scanner may have different sensitivities in different environments and to different fluorescence sources and it is necessary to eliminate these differences to generate high quality dual channel images. By using *Histogram*, the user can easily measure such differences and balance the dual channel data by choosing *Laser Power* and *PMT Gain* settings.

Here are some examples describing how to use *Histogram* according to the microarray performance.

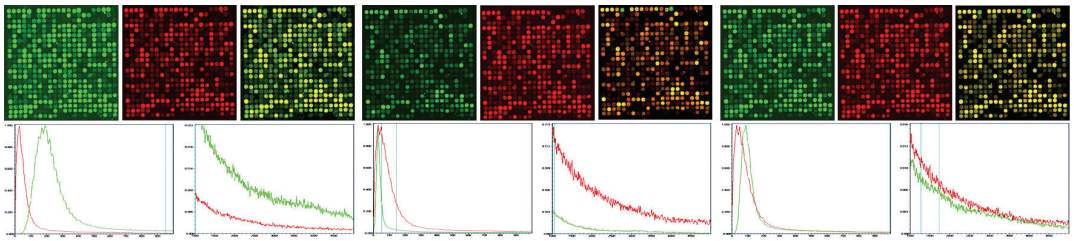


Figure A demonstrates a poor adjustment of the *Laser Power* and *PMT Gain*, because the Cy3 channel is set too high and the signals in the range 1000 to 5000 are out of balance. The Cy3 are higher than Cy5 and the composite image appears green.

Figure B shows another poor adjustment of the *Laser Power* and *PMT Gain*, because the Cy5 channel is set too high and the signals in the range 1000 to 5000 are out of balance. The Cy5 are higher than Cy3 and the composite image appears red.

Figure C shows a better adjustment of the *Laser Power* and *PMT Gain*. The signals in the range 1000 to 5000 are well balanced, and the composite image appears yellow.

Chapter 5 System Maintenance

5.1 Overview

As a precision optical instrument, the LuxScan™ 10K scanner needs routine maintenance for quality service. New versions of the application software with improved functions may be released in the future.

No user maintenance requiring removal of the protective housing is permitted. If the protective housing needs to be opened, please contact CapitalBio technical support

Warning:

Users are not allowed to remove the outer protective housing at any time.

5.2 Key Issues for Hardware Maintenance

Note:

- Use a soft cloth to clean the surface of the scanner. Do not use liquid cleaners.
- Do not keep the lasers on for more than 12 hours.
- Do not power on and off the scanner frequently. The power off time should be longer than 30 seconds before powering on again.
- The scanner must be placed on a leveled bench.
- Only insert a completely dry chip into the scanner.
- Follow the instructions in this manual to install and use the scanner.
- Do not shake the scanner while moving it from one location to another.

5.3 Software Re-installation and Upgrade

Re-installing LuxScan™ 3.0 software will not damage any scan images or saved protocols. LuxScan 3.0 and LuxScan 2.x can co-exist in the same system.

Chapter 6 Troubleshooting

6.1 Overview

In this chapter, we provide solutions to frequently encountered problems for using the LuxScan™ 10K scanner. If these solutions do not solve your problems, please contact CapitalBio technical support, see "Where to Get Help" in the Preface for details.

6.2 Problems and Solutions

6.2.1 Scanner Can Not Be Powered On

- Check the power supply system: the appliance inlet, the mains connector, power cable, the mains plug and the fixed mains socket-outlet.
- Disconnect the power cable and check the fuse-holder at the rear panel of the scanner, replace the fuses if necessary.

6.2.2 Scanner Does Not Response to Software Commands

- Check the USB cable and connections.
- Check the LEDs on the scanner panel, and make sure the scanner is ready.
- Restart the software. If the software can not find the scanner, a warning message will appear.

6.2.3 No Image Produced After Scanning

- Check whether the chip has been properly inserted.
- Check whether the selected laser wavelength matches the fluorophore on the chip.
- Check the Laser Power and PMT Gain settings.

6.2.4 Insufficient System Memory

- There may be too many images opened. Close some of them to free the memory.
- There may be too many programs running. Close some of them to free the memory.
- This problem can be solved by expanding the virtual memory of the computer workstation.

6.2.5 USB Error

- Check the USB cable connection.
- Power off the scanner and exit the software. Restart the system after 30 seconds.

6.2.6 Scanner Not Found

- Make sure the scanner is powered on and is properly connected to computer workstation.
- Make sure the USB device icon is displayed in the Microsoft Windows® taskbar.

6.2.7 Can Not Select the Preferred Laser in Scan

- LuxScan™ 10K-B has only one laser in the instrument.
- Make sure the laser is switched on.

6.2.8 System Self-Test Failed or LED Indicates System Error

- Shut down and restart the system, run self-testing again.
- If the error still exists, please contact CapitalBio technical support.

6.2.9 Failed to Open Image File

- Please ensure your file is in the appropriate format. The software can only read 16-bit standard TIFF format image files.

6.2.10 Failed to Auto-align

- The value of the column or row spacing between a *circle* and its associated *spot* is two and a half times less than the average radius of the *spot*s.
- The value of the column or row spacing defined by the user is longer or shorter than the actual spacing between the *circle* center and its associated *spot* center.
- There is a difference (more than about 20%) between the radius of a *circle* and its associated *spot*.

Shortcut Keys

Command	Short-cut Key
Open	Ctrl + O
Save	Ctrl + S
Select All Objects	Ctrl + A
Copy Selected Objects	Insert
Delete Selected Objects	Delete
Zoom In Mode	Z
Zoom Out Mode	X
Move Mode	V
Zoom to Fit Image	F3
Zoom to Fit Selected Objects	F4
Create *ROI*	A
***ROI* Mode**	R
Load Array List	G
Create *Blocks*	T
***Block* Mode**	B
***Circle* Mode**	C
Auto Align Selected *Blocks*	F5
Align All *Blocks*	F6
Extract Data	F7
Histogram	F10
Scatter Plot	F11
Move Selected Object	Arrow Keys
Select Next / Previous *Block*	< and >
Change *Circle* Size	Page Up and Page Down
User Manual	F1

Specifications

Specifications	Description
Chip Dimensions (mm)	(25.40 ± 0.25)×(76.20 ± 0.50)×(1.00 ± 0.10)
Scan Area (mm)	22 × 72
Excitation Wavelengths (nm)	532 and 635
Emission Wavelengths (nm)	570 and 675
Scan Resolution (μm)	5, 10, 20 and 40
Sensitivity (Fluorophore/μm^2)	≤ 0.1
Scan Speed	8 minutes / channel at 10μm resolution
Dynamic Range	> 10^4
Digital Resolution	16 bits
File Format	TIFF
Operating System	Microsoft Windows® 2000 (with Service Pack 4), or Microsoft Windows® XP (with Service Pack 1 or higher)
Dimensions (mm)	540 (L) ×360 (W) × 220 (H)
Weight (kg)	40 (88lb)
Power Supply	120V~ / 230V~ , 50 / 60 Hz

Glossary

Term	Definition
System Administrator	A user with the highest privileges to access the computer workstation.
PMT	Photomultiplier Tube. A light sensitive vacuum tube that controls the current based on the amount of light received by the tube. A PMT with high gain has a high current to light ratio.
Focus Position	Focus Position is positioned above the spotted surface of the chip.
PMT Gain	The ratio of the current to the light energy for the PMT output. The higher the PMT Gain, the weaker the light needed for a given current.
Resolution	The pixel size. The maximum resolution of LuxScan™ 10K scanner is 5μm.
Scan Area (ROI)	A rectangular region on the chip to be scanned. You can define several Scan Areas for one chip.
Scan Protocol	A set of scan areas and scan parameters for the settings of laser channels, fluorescence source, laser power, PMT Gain and focus position, etc.
Color Palette	A range of colors assigned to image pixels based on the intensity value of the pixel, allowing the user to render a gray image to a pseudo-color image for better visual effect.
Map Range	Control the range of gray scale in which the palette colors will show as a gradient.
Laser Power	As the laser is the energy source of the fluorescence, the higher the Laser Power, the more fluorescence photons the scanner will detect. Increasing the Laser Power will increase the intensity of the image pixels. The range of Laser Power is from 1% to 99%.
Red Laser	The 635nm wavelength laser used in LuxScan™ 10K scanner for specific fluorophores, e.g. Cy5, BODIPY 630/650, Alexa 633, Alexa 647, BODIPY 650/665, Alexa 660, Cy5.5, Alexa 680.
Green Laser	The 532nm wavelength laser used in LuxScan™ 10K scanner for specific fluorophores, e.g. Cy3, Alexa 532, POPO-3, PO-PRO-3, Alexa 546, Alexa 555, Alexa 568, Cy3.5.
File Format	The format of the image files. TIFF is the standard format for scanned images. Other formats e.g. JPEG, BMP, RAW formats are also supported for pseudo-color images.

Term	Definition
TIFF	Tagged Image File Format (TIFF or TIF) is the default file format for the scanned images. TIFF is the standard uncompressed image file format. The LuxScan™ 10K application software can save and read scanned images in the TIFF format.
JPEG	Joint Photographic Experts Group (JPEG or JPG) is a file format of loss compression. That is, the file stored in this format will lose some information without affecting the visual features. The LuxScan™ 10K application software can save and read pseudo-color images in the JPEG format.
BMP	Bit Map File Format (BMP) is the bit map file format built into Microsoft Windows® operating system. That is, the operating system uses this format to store bit maps. The LuxScan™ 10K application software can save and read pseudo-color images in the BMP format.
LED	Light-emitting diode.
Histogram	A histogram is a plot, which represents the frequency distribution of all pixel intensities in dual channel images.
Array	An array is a two dimensional matrix of spots on a microarray chip.
Background	A local background region is a circular region that surrounds the spot of interest. The circular region has a radius of two to four times the radius of the current circle. All the pixels belonging to the adjacent circles, or the circle of interest, are excluded.
Background CV	The Coefficient of Variance (CV) of the local background pixel values.
Background Intensity	The background intensity of a spot is the median or mean of the intensities of all the pixels in the background region.
Background Mean	The mean of the values of all the local background pixels.
Background Median	The median of the values of all the local background pixels.
Background SD	The Standard Deviation (SD) of all the local background pixel values.
Block	A block is a collection of circles drawn on an image. The collection of all the blocks on a microarray chip is called a grid, or a block group.
Circle	A circle is a circular region defined on an image within which software calculates spot intensity based on the pixel values in the region.
Column Spacing	The distance between the centers of two adjacent spots in a row of a subarray.

Term	Definition
Grid	The collection of all the blocks on a microarray chip image.
Pixel	Images are made up of a two-dimensional array of picture elements, or pixels. The resolution of an image is determined by the size of its pixels. For example, a 5μm pixel-size image is of higher resolution than a 10μm pixel-size image.
Pixel Value	The raw value of a pixel. The pixel value ranges from 0 to 65,535 for a fluorescent scanned TIFF format image.
Row Spacing	The distance between the centers of two adjacent spots in a column of a subarray.
Spot	A spot is a fluorescent element on a microarray chip.
Spot CV	The Coefficient of Variance (CV) of all the pixel values in a spot region.
Spot Intensity	The median or mean value of all the pixels in a spot region.
Spot Mean	The mean of the values of all the pixels in a spot region.
Spot Median	The median of the values of all the pixels in a spot region.
Spot SD	The Standard Deviation (SD) of all the pixels in a spot region.
X	The X-coordinate, in μm, of the circle center associated with the spot, where (0, 0) is the top left corner of the image.
Y	The Y-coordinate, in μm, of the circle center associated with the spot, where (0, 0) is the top left corner of the image.
LSR	LuxScan Result File is a result data file format defined by LuxScan™. An LSR file contains the information about image analysis and the data extracted from each individual circle. Files in this format can be viewed and processed by Microsoft Excel.
ASR	AlphaScan Result File is a result data file format defined by AlphaScan. An ASR file contains the information about image analysis and the data extracted from each individual circle. Files in this format can be viewed and processed by Microsoft Excel.
GAL	Gene Array List File Format.
XAL	Extended Array List File Format.

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